

HISTOCHEMICAL STUDIES ON THE ACANTHOCEPHALA:
A CONTRIBUTION TO HELMINTH METABOLISM

BY
WILBUR LEWIS BULLOCK

B. S., Queens College, 1942
M. S., University of Illinois, 1947

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY
IN THE GRADUATE COLLEGE OF THE
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HISTOCHEMICAL STUDIES ON THE ACANTHOCEPHALA: A CONTRIBUTION TO HELMINTH METABOLISM

In the present investigation some of the most recent histochemical techniques have been used to localize substances of importance in the metabolism of the Acanthocephala. The substances studied include glycogen, lipoids, alkaline phosphatase, lipase, tryptophane, tyrosine, and arginine. In addition some cytological studies were made in an attempt to relate the Golgi apparatus to some of these substances.

MATERIALS AND METHODS

The Acanthocephala used in this investigation were *Echinorhynchus coregoni*, *E. gadi*, *Pomphorhynchus bulbocolli*, *Neoechinorhynchus cylindratus*, and *N. emydis*. All but the last named are parasites of fishes. Fish viscera were obtained from a Chicago fish market. The parasites were removed from the host intestines and placed in the desired fixative. Since nothing is known of the physiological condition of the host save that food had not been taken for several days to several weeks, it is concluded that the results of this study probably show the effects of starvation. Such a condition is also true of *N. emydis* which was taken from turtles supplied by a dealer.

Glycogen was studied by means of the Baur-Feulgen technique with sections digested in saliva as controls. General distribution of lipid material was investigated with osmic acid, Sudan IV, and Sudan Black. Phospholipid was localized by the use of bichromate either in Champy fixed material or by the application of the Smith-Dietrich test and the McManus technique. Cholesterol distribution was studied with the Schultz modification of the Liebermann-Burchardt test. The localization of alkaline phosphatase was investigated by means of the Gomori technique. The substrate used was sodium glycerophosphate at a pH of

9.0-9.5. Gomori's lipase technique was applied to acanthocephalan material using Tween 60 as a substrate at a pH of 7.0-7.4. Tryptophane was localized by means of the aldehyde test which produced a violet color indicating the presence of the indole ring. The distribution of tyrosine was estimated by the difference between tryptophane as indicated by the aldehyde test and the sum of tryptophane and tyrosine as indicated by the xanthoproteic test. Arginine was localized by means of Sakaguchi's test. Localization of the Golgi element was studied by the DaFano technique while mitochondria were stained by the Champy-Kull method.

RESULTS

1. Glycogen was found largely in the muscles and the subcuticula of all species studied although all organs contained some. Those species with a thicker subcuticula, such as *E. coregoni*, showed larger glycogen stores indicating a potentially greater carbohydrate reserve than in those forms with a thinner subcuticula, such as *N. emydis*. Evidence was also obtained for existence of a polysaccharide other than glycogen in the embryonal-kern region of the embryos.

2. Fat distribution approximately paralleled that of glycogen. On the basis of the differential tests it was concluded that the subcuticula of the praesoma and the lemnisci contained mainly triglyceride, fatty acid, or both. The muscles also contained large amounts of this type of fat in addition to phospholipid. The subcuticula of the trunk contained largely phospholipid. Cholesterol was also localized here.

3. Lipase was observed in the subcuticula of the trunk and the lemnisci.

4. Alkaline phosphatase was limited to the subcuticula of the trunk. The heaviest concentration was in the outer layer immediately below the cuticula.

5. Tryptophane was most concentrated in the subcuticula

of the praesoma and the lemnisci. The subcuticula of the trunk and possibly the muscles also showed evidence of the presence of this amino acid.

6. Tyrosine appeared generally distributed throughout the acanthocephalan body.

7. Arginine was found in the muscles and in the embryonal-kern.

8. Evidence for the existence of a Golgi apparatus was found in the outer layer of the subcuticula in the form of minute granules.

9. Mitochondria were found sparsely distributed throughout the inner layer of the subcuticula of the trunk.

10. Species of the genus *Neoechinorhynchus* showed generic peculiarities in that neither phosphatase nor lipase could be demonstrated by methods invariably successful on other forms.

CONCLUSIONS

1. The subcuticula of the *Acanthocephala* is an important site of physiological activity.

2. Glycogenesis probably takes place in the subcuticula but the site of the major part of glycolysis is unknown.

3. If phosphatase is necessary for the transformation of carbohydrate to fat then the subcuticula is the only tissue in which this change can occur. Thus the theory that the production of fatty acid as the main end-product of carbohydrate metabolism on the basis of parallel distribution of fat and carbohydrate is shown to be unsound.

4. The heavy concentration of phosphatase in the outer layer of the subcuticula functions in the absorption of carbohydrate and probably of fat. This region of phosphatase activity may be associated with the Golgi zone.

5. Fat hydrolysis and possibly synthesis under the control of lipase also occurs in the subcuticula of the trunk and to some extent in the lemnisci.

6. The subcuticula of the praesoma and the lemnisci probably represent a physiological unit for the direct absorption or excretion of triglyceride or fatty acid.

7. Distribution of tryptophane and tyrosine furnish good examples of tissue protein specificity.

8. Distribution of arginine in the muscles conforms to the role of this substance in the contractive mechanism of invertebrates in general.

VITA

Wilbur Lewis Bullock was born in Brooklyn, New York, on March 8, 1922. He attended elementary school in New York City and graduated from John Adams High School in 1938. He attended Queens College, Flushing, New York, receiving the B. S. in Biology in February, 1942. Following graduation he worked in industry as a chemist for eight months. He then accepted a teaching assistantship at New York University which he held until entering the army in July, 1943. He served in Europe as medical aid man with a combat engineer battalion. At the end of the war he became an assistant instructor in Zoology at the Biarritz American University in the historic French town on the Bay of Biscay.

Soon after being discharged in December, 1945, he was admitted to the Graduate School of the University of Illinois, from which he received the M. S. in Zoology in February, 1947. He held a teaching assistantship at the University of Illinois from September, 1946, to June, 1947. At that time he became a Fellow in Zoology. He is a member of the following scientific societies: Beta Lambda Sigma, Phi Sigma, and Sigma Xi.



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